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BACTERIAL PROFILE AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERNS ISOLATED FROM PATIENTS WITH EXTERNAL OCULAR INFECTIONS AT BORUMEDA HOSPITAL, NORTHEAST ETHIOPIA

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LIST OF ABBREVIATIONS

ATCC	American Type Culture Collection
BH	Borumeda Hospital
CoNS	Coagulase-negative *Staphylococcus*
MDR	Multi-drug Resistant
MRSA	Methiclline Resistant *Staphylococcus aureus*

ABSTRACT

Background: Ocular infections caused by different groups of bacteria are important public health problems worldwide. The situation is more worsened by the alarmingly increasing rate of antibiotic resistance.

Objectives: The aim of this study was to assess the magnitude of the bacterial isolates, associated risk factors and their antimicrobial susceptibility patterns isolated from patients with external ocular infection at Borumeda Hospital, Northeast Ethiopia.

Methods: A cross-sectional study was conducted at Borumeda Hospital from February to May, 2014. A systematic random sampling technique was used. Socio-demographic data and possible risk factors were collected using structured questionnaire. External ocular specimens were collected according to standard operational procedure. After collection, specimens were inoculated on MacConkey agar, chocolate agar, blood agar and Mannitol Salt Agar. Presumptive bacterial colonies were isolated using a series of biochemical tests based on their Gram reaction. Kirey Bauer disk diffusion method was used to determine the antimicrobial susceptibility pattern of the isolates. Data were entered and analyzed using SPSS version 20. Descriptive statistics and logistic regression analysis was used to determine dependant and independent variable. P value <0.05 were considered as statistically significant to all test.

Results: Among 160 patients, males were 94(58.8 %). The mean age of study participants was 55.11 years. Majority was 95(59.4%) culture positive for 89 (93.7%) Gram positive and 6(3.8%) was Gram negative. Coagulase negative *Staphylococci* were the predominant isolate 51(31.9%) followed by *S. aureus* 21(13.1%) and *S. pneumoniae* 10 (6.2%). Total multidrug resistance shows in 48 (50.5 %) of the isolates. All Gram positive isolates were susceptible for vancomycin but 60(46.1%) of them showed resistance against amoxicillin. Resistance for tetracycline, norfloxacylin, ceftriaxon and ciprofloxacin 4 (66.7 %) for each, was observed among Gram negative bacterial isolates. Multivariate analysis shows the predisposing risk factor were no statistical significant association with dependant variable.

Conclusion: The prevalence of bacterial isolates in external ocular samples was high in the study area and the dominant bacterial isolate was coagulase negative staphylococci. Amoxicillin resistance among Gram positive bacterial isolates was exceptionally high. Although the number of Gram negative bacterial isolates were small, drug resistance was comparatively significant. Therefore, identification of ophthalmic bacterial etiological agent and conducting drug susceptibility test could reduce drug resistance and increase cure rate during treatment.

Key words: *Bacterial isolate, External ocular infections, Susceptibility pattern, Dessie*

1. INTRODUCTION

1.1 Background and Statement of the Problem

Although relatively impermeable to microorganisms, infection within the eye can result from trauma, ocular surgery or systemic disease. An infection in the eye does not respect anatomical boundaries may occurred in any area and will spread to affect several different anatomical structures but the conjunctiva, lid and cornea are more frequently infected area (1). Infection of the eye can lead to loss or impairment of visual function with clinical manifestations reported in the world are conjunctivitis, scleritis, keratitis, blepharitis, canaliculitis, dacryocystitis; deeper infections such as orbital cellulitis, preseptal cellulitis, necrotizing fascitis and intraocular infections such as uveitis, endophthalmitis, panophthalmitis (2).

Ocular infection is an important public health problem in the world and the morbidity can vary from self limiting, trivial infection to sight threatening, caused by bacteria, fungi, virus and protozoa (3). Pathogenic microorganisms cause diseases to the eyes due to their virulence and hosts reduced immunity. Just as the blind spot is neglected by the brain, about 45 million people in the world who are blind are largely neglected by medical science and technology, and by the caring professionals; of this 80% are preventable. The situation in Ethiopia is similar with prevalence of blindness being about 1.5% and an estimated around 1.05 million blind people (4).

Bacteria are the major causative agents that frequently cause infections in the eye. The common bacterial isolates associated with ocular infections include coagulase negative *Staphylocci* (CoNS), *Staphylococcus aureus, Streptococci* spp, Gram negative cocco bacilli and Gram negative bacilli (5, 6).

A serious bacterial eye infection that threatens the cornea and intraocular area is an ophthalmic emergency that needs immediate treatment (7, 8). Management of bacterial eye infection may involve treatment with broad spectrum antibiotics. This is most at times before pathogen identification and antibiotic susceptibility tests are available. This indiscriminate use of antibiotics has led to the development of resistance to many commonly used antimicrobial medications. This development of antimicrobial resistance are influenced by characteristics of pathogens, antibiotic prescribing practices include overuse of antibiotics for systemic infection as well as overuse of topical antibiotics in the eye, misuse of antibiotics for viral infections, extended duration of therapy and current globalization and migration of populations (9, 10).

The knowledge of the infection, epidemiology and the antibiotic resistance patterns is essential to guide optimal empiric treatment in critically ill patients. Antibacterial susceptibility patterns and bacterial spectra vary geographically, highlighting the importance of local surveillance data. In industrialized countries, these data are available at regional, national and international levels; for instance as provided by the European antimicrobial resistance surveillance system database (11, 12). However, current surveillance data are scarce in Africa (11, 13). As there is a worldwide problem regarding the emergence of bacterial resistance towards topical antimicrobial agents, which increases the risk of treatment failure with potentially serious consequences leads to more prevalent in Africa (14). Therefore, up to date information is essential for appropriate antimicrobial therapy and management of ocular infections (15).

In the study area as well as in Ethiopia there is a scarcity of published data on the spectrum of etiologic agents responsible for external ocular infections. Thus the aim of this study was to assess the magnitude of the bacterial isolates, possible risk factor and their antimicrobial susceptibility patterns isolated from patients with external ocular infection at Borumeda Hospital, Northeast Ethiopia.

2. LITERATURE REVIEW

2.1 Prevalence and Etiology of Ocular Infection

The prevalence of external ocular infections that are caused by bacterial organisms varies with the age of the patient, children and elderly patients are more susceptible to infection by bacteria than young and middle-aged adult, (16) climate, and geographical variation: living in rural or in city areas, in western, or in developing countries also spread differently (17).

Corneal diseases, specially infective keratitis, are a major cause of vision loss and blindness second only to cataract (18, 19). In United Kingdom isolates including coagulase negative *Staphylococcus* (CoNS) (30%), *Pseudomonas aeruginosa* (23%), *Staphylococcus aureus* (14%), Enterobacteriaceae (14%), and *Streptococci* species (13%) were the main causative etiology for bacterial keratitis (20). Study conducted in Paulo, Brazil, showed that 46-69% of keritites were caused by bacteria pathogens and reported also that CoNS infection was predominant (21).

Staphylococcus epidermidis is the main organism among the CoNS. It is part of the normal flora of the skin and conjunctival sacs but may act as a pathogen causing fatal infections which may have a significant incidence especially in the immune compromised host like diabetes and acquired human immunodeficiency virus (AIDS) patients (22, 23).

Gram positive *cocci*: *Streptococcus pneumoniae*, *Hemophilus influenza*, Group A *Streptococcus* were the main pathogens isolated form adult conjunctivitis (24). *Streptococcous viridans* was associated with conjunctivitis in patients aged less than 1 year (25). The most common causes of purulent conjunctivitis are *Neisseria gonorrhea* or *Staphylococcus* species infection (26, 27). Contact lens wearers more likely develop Gram negative conjunctivitis (28).

Bacteriologic and plasmid analysis of etiologic agents of conjunctivitis in Lagos, Nigeria showed that Gram positive cocci isolate comprising *Staphylococcus aureus* and CoNS species accounted for 50.3% of conjunctivitis cases, followed by Gram positive bacilli, Gram negative bacilli and Gram negative cocci. *Corynebacterium* species was the most commonly

isolated Gram positive bacilli accounting for 16.1% of conjunctivitis cases. *Pseudomonas aeruginosa* topped as the most commonly isolated Gram negative bacilli. Other Gram negative bacilli in order of their isolations were *Escherichia coli*, *Proteus* species, *Klebsiella* species, and *Enterobacter aerogenes*. From Gram negative cocci *Moraxella* species was the only isolate. Further analysis of the complexity of infections showed that, polymicrobial infections caused by two pathogens and three or more pathogens of conjunctivitis specimens screened (29). In Uganda from conjunctival samples 45.8% cases were culture positive. The most common organisms identified were CoNS and *Staphylococcus aureus* (30).

Even if there is extremely little literature about the prevalence or incidence of blepharitis, ophthalmologists and optometrists surveyed that, it is commonly seen in 37% - 47% of their patients. The etiology of blepharitis can be bacterial growth commonly caused by *Staphylococcus aureus* and sebaceous gland, or meibomian glands malfunction (31).

Gram positive organisms: *Streptococcus pneumoniae*, *Streptococcus pyogens*, *Staphylococcus aurous*, *Streptococcus viridans* were the most commonly isolated from lacrimal abscess accounting for 62.6%. *Hemophilus influenzae* was the commonest Gram negative isolate (32). A comparative study in India shows that proportions of *Staphylococcus aureus* and *Pseudomonas* species are higher in causing acute dacryocystitis, (33), while the proportion of CoNS is higher in chronic dacryocystitis in most of the country. But in Malaysia and Ethiopia *Streptococcus pneumoniae* was the commonest identified Gram positive organism (32,34).

Trachoma is the leading infectious cause of blindness worldwide. In 2003, World health organization estimated that 84 million people were suffering from active trachoma, and 7.6 million were severely visually impaired or blind as a result of trachoma in the world (35). It is caused by infection with *Chlamydia trachomatis* serovars A, B, Ba and C and is characterized by inflammatory changes in the conjunctiva in children with subsequent scarring, corneal opacity and blindness in adults. It is associated with poverty in environments with inadequate sanitation, poor personal hygiene and poor water supply and is now largely confined to developing countries, particularly in Africa and Asia (36).

In North India children *Chlamydia trachomatis* antigen positivity was varied between 22-28 % (37). In Ethiopia trachoma was found to be the leading cause of ocular morbidity among children aged 1-9 years with prevalence of 33.7% - 40.14% (38, 39). The national prevalence of *trachomatous trichiasis* is 3.1% with the highest prevalence in Amhara regional state (5.2%). Over 9 million 1-9 year old children live with active trachoma, and 1.3 million people in 15 years and older have *trachomatous trichiasis* (39).

In general, the prevalence of bacteria caused external ocular infection in Ethiopia ranges 47.4% - 74.7% culture positive for different types of bacterial pathogens (40, 41). The most common etiologic agent isolates were, *Staphylococcus aureus*, followed by *Streptococcus pnuemoniae*, CoNS, *Hemophilus influenza* for Gram positive and *Psuedomonas* species for Gram negative (40, 41); other study show *Escherichia coli* was the most predominant Gram negative isolate (42),then *Hemophilus aegyptius*, *Klebsiella pneumonia*, *Moraxella* species, *Neisseria meningitides* and others (40, 41).

2.2 Antimicrobial Susceptibility Pattern of Pathogens in Ocular Infections

Local application of antibacterial compounds to the eyes may lead to bacterial resistance in bacterial isolates from the eyes (43). Upon exposure to an antibacterial agent, bacteria with these types of natural resistance mutations will be able to reproduce at a quicker rate than the vulnerable bacteria. The problem of antimicrobial resistance shows no boundaries. But the threat of antibiotic resistance is growing at an alarming pace more rapidly in developing countries (44).

There are low resistance rates of *Pseudomonas aeruginosa* and *Staphylococcus aureus* to ciprofloxacin in isolates from Australia. Isolates from the Indian subcontinent are more commonly resistant to ciprofloxacin, with resistance rates of greater than 20 % reported. Data from United States of America and Europe indicate that if the *Staphylococcus aureus* is a methicillin resistant strain (MRSA) then resistance to ciprofloxacin increases, often to greater than 80% of isolates. Resistance to gentamicin and cephalosporins is also generally low in isolates from Australia (45).

In Brazil, between 1989 and 1992 no resistance to gentamicin was reported in *Streptococcus pneumonia*. However, resistance to gentamicin was reportedly 42.3% in 1997 which increased to 56% in the year 2000 (46). Similarly in South Florida MRSA showed significant increase in 2007 ranged from 27.4 to 62.4% in prevalence. There were 2- and 3-fold increases in resistance of Gram positive organisms to erythromycin and ciprofloxacin, respectively. Gentamicin showed good sensitivity toward Gram positive pathogens (47).

Even if a very few available reports on vancomycin resistant *Staphylococcus aureus* ocular infections; resistance to most groups of antibiotics is increasing with resultant decline in the effectiveness of many commonly used topical antibiotics (48). *Pseudomonas* species tops the list of challenging organisms to treat because of high prevalence of resistant strains. Multidrug resistant (MDR) *Pseudomonas aeruginosa* have been reported from keratitis and endophthalmitis patients leaving no choice other than to use piperacilin/tazobactum or imipenem only for the treatment of such cases (45).

In Ethiopia, study conducted in Jimma University Specialized Hospital on bacteriology of ocular infections and antibiotic susceptibility patterns reported that majority of Gram positive cocci were susceptible to ciprofloxacin and vancomycin and Gram negative isolates to amikacin and ciprofloxacin (41). On the other hand, reports from Gondar showed that there is high incidence of MDR problems in ocular pathogens that ranges 77.3% of bacterial isolates to the commonly prescribed antibiotics (42), leaving ophthalmologists with a very few choices of drugs for the treatment of ocular infections (14).

3. SIGNIFICANCE OF THE STUDY

Vision impairments and blindness present substantial social and economic burdens on individuals and society including significant suffering, disability, loss of productivity and diminished quality of life for millions of people.

Clinical presentations alone are not diagnostic and a microbiological analysis with cultures and antimicrobial sensitivities is mandatory for the selection of a specific antimicrobial therapy. The clinical importance of external eye infections has been reported in some studies in Ethiopia, with shortage of studies which have been supported by laboratory diagnosis and the etiologic agents are scarcely known. Therefore treatment is empirically given with broad spectrum antibiotics. This might results in a global increase in resistance among both Gram positive and Gram negative bacteria to common antibiotics used to treat ophthalmic infections and increase in morbidity, mortality and cost of health care.

So far there is no previous data or study showing etiology of microbial external ocular infections and sensitivities pattern in the study area, this study attempted to identify the magnitude of the etiologic agents and microbiological characteristics of microbial external ocular infections and antimicrobial susceptibility patterns, which has an important public health implication to gives a base line data for clinicians, health sector administrators, concerned bodies and researchers.

4. OBJECTIVES

4.1. General Objective

The general objective was to assess the magnitude of bacterial profile, antimicrobial susceptibility patterns and associated factors of bacterial profile isolated from patients with external ocular infection at Borumeda Hospital, Northeast Ethiopia.

4.2. Specific Objectives

- To determine the prevalence of bacterial pathogens from patients with external ocular infection.
- To determine the antimicrobial susceptibility patterns of bacterial isolates from patients with external ocular infection.
- To identify possible factors associated with the bacterial external ocular infections.

5. MATERIALS AND METHODS

5.1 Study Area

The study was conducted at Borumeda Hospital in Dessie town. Dessie is capital city of South Wollo Zone in Amhara regional state, North East Ethiopia, located 401 km far from Addis Ababa. In this town there are 16 governmental health institutions (1 referral hospital, 1 primary hospital, 8 health centers and 6 health posts), and one regional health research laboratory where culture and sensitivity tests were performed. One of the primary hospitals is Borumeda Hospital, where this study was conducted. Borumeda Hospital has 57000 patient flows per year, 110 beds for in patient and serving for one million people that are found around the community.

5.2 Study Design and Period

A cross-sectional study was conducted from 15th of February to the 15th of May 2014.

5.3 Population

5.3.1 Source Population

- All patients with external ocular infection attending Borumeda Hospital.

5.3.2 Study Population

- All patients with external ocular infection attending Borumeda Hospital during the study period.

5.4 Exclusion Criteria

- All Patients with external ocular infections, who had treatment with antibiotics in the last 7 days.
- All Patients with external ocular infections: who performed ocular surgery within 7days.
- All Patients with external ocular infections: who developed severe ocular trauma within 7 days.

- All Patients with external ocular infections who developed, keratitis, endophthalmitis and allergic conjunctivitis.

5.5 Inclusion Criteria

- All patient who develops external ocular infections included in this study

5.6 Operational Definition

- **External ocular infections**: infections in the external eye structures include the eyelids and surrounding tissues, conjunctiva, lacrimal apparatus, cornea, and anterior chamber.
- **Frequency of face washing**: more frequent (washing of face two or more times per day), frequent (washing of face once per day) and less frequent (washing of face occasionally per a week) using water.

5.7 Variables

5.7.1 Dependent Variables

- Bacterial isolates

5.7.2 Independent Variables

- Age, sex, occupation, education, residence
- Previous history of ocular trauma
- Frequency of face wash
- History of past ocular surgery
- Systemic disease

5.8 Sample Size and Sampling Technique

Sample Size

The sample size were determined using single population proportion formula

$$n = (Z\alpha/2)^2 \frac{P(1-P)}{d^2}$$

Where; n= the minimum sample size; Z= Standard normal distribution value at the 95% CI, which is 1.96; α = level of significance; d= margin of error, taken as 5%; P= 75% prevalence ocular infection taken from previous similar study conducted in Jimma university specialized hospital.

=1.96x1.96 x 0.75(1-0.75)/0.05x0.05

= 288 people

From the above sample size calculation, n=288 were the sample size. However, the average daily flow rate of external ocular infection in the eye clinic of the hospital is 5. The study period were taking 3 months with 22 working days each month. Therefore there were a total N= 330 patients visiting the clinic during the study period. Since expected numbers of patients coming to eye clinic are less than 10,000, correction formula was used to get the final sample size.

$$nfinal = \frac{n}{1 + \frac{n}{N}}$$

$$nfinal = \frac{288}{1 + \frac{288}{330}} = 288 \div 2 = 144$$

When 10% non response rate was considered, the final sample size becomes 159.

Sampling Techniques

Systematic random sampling technique was used to select study subjects coming to the eye clinic during the study period. Hence, to get 160 samples size, every k=N/n (330/160=) 2nd client visiting the center was included by considering every 2nd client until the sample size reached. The 1st client was selected by lottery method by writing number less than k value. A patient who starts treatment with antibiotics in the previous day was excluding from the study.

5.9 Data Collection and Laboratory Investigation

5.9.1 Socio-demographic data collection

After completed the consent form, Socio-demographic data and possible risk factor for bacterial external ocular infection were collected by trained optometrist from each study participants by using pre-tested structured questionnaire.

5.9.2 Specimen Collection and Laboratory Investigations

I. Specimen collection

After detailed ocular examinations using standard techniques external ocular specimens for culture and smear were collected using moistened by saline, sterile cotton swab by gently swabbing the eye, the lower conjuctival sac and lid margins. Purulent material in dacryocystitis was collected by everted puncta then applying pressure over the lacrimal sac area from more infected eye at outpatient department (OPD) of BH. Swabs of ocular samples from patients were aseptically obtained from ocular infection sites before the eye were cleaned with an antiseptic solution, topical anesthetic and antibiotic used. The swab was immersed in 2ml of brain heart infusion and transported, using cold box, to Dessie Regional Laboratory for investigation (49, 50).

II. Isolation and Identification of Bacteria

The collected specimens were inoculated on to MacConkey agar (Oxoid Ltd Basingstoke, Hampshire, UK), Manitol Salt Agar(Oxoid Ltd) , Blood agar (Oxoid, LTD) incubated at 35°C-37°C for 24hrs on the other hand, the inoculated chocolate agar plates were incubated in addition of 5% CO2 atmosphere. All the plates were initially examined for growth after 24 hours; and cultures with no growth were incubated for further 48 hours. After obtaining pure colonies, further identification were conducted by using the standard biochemical tests after differentiating using Gram's staining (49). Gram negative rods were identified by performing a series of biochemical tests which include triple sugar iron agar, indole, Simon's citrate agar, lysine iron agar, urea, mannitol and motility. Gram positive cocci were identified based on their Gram reaction, catalase, coagulase, bacitracin and optochin test results (49).

III. Antimicrobial susceptibility testing

The antimicrobial susceptibility test of the isolates was performed according to the national committee for clinical laboratory standards (NCCLS) method using Kibry-Bauer disk diffusion test on Muller-Hinton agar. Biochemically confirmed isolates were suspended in a nutrient broth and incubated for 30 minutes to make it comparable with 0.5% McFarland standard. After incubation a sterile cotton swab was dipped in to the suspension and bacteria were inoculated on to the Muller-Hinton agar (Oxoid CM0337 Basingstoke, England).

The standard antibiotic disks used from Oxoid (UK) were gentamicin 30 µg, ampicillin 30 µg, amoxicillin 30 µg, and cefoxitine 25 µg, penicillin 10U, vancomycin 30 µg for Gram positive and gentamicin 30 µg ciprofloxacin 5µg, tetracycline 30µg, doxycycline 30µg, were used for Gram negative bacteria. Antibiotic discs were placed by using disc dispenser on the agar surface in such a way that each disk were placed at least 24 mm away from each other to avoid the overlapping zone of inhibition. After the disk is placed, the plates were allowed to stand for 15 minutes for the antibiotic to dissolve in the media and the plate was incubated for 24 hrs at 35°C. Results were interpreted as sensitive, intermediate and resistant after measuring the zone of inhibition and comparing with the standards (49).

5.9.3 Quality Control

The questionnaire was prepared in English and translated to Amharic and then back translated to English for consistency. The questionnaire was pretested on ten study subjects in Dessie Referal Hospital eye clinic and amendments were made.

All specimens were collected according to the standard operating procedure. The sterility of culture media was ensured by incubating 5% of each batch of the prepared media at 37^{o}c for 24 hours. Performances of all prepared media were also checked by inoculating known bacterial strains such as *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923) and *P. aeruginosa* (ATCC 27853) and *Neisseria gonorrheae* (ATCC 49226) were employed as strains of quality control for the antimicrobial susceptibility test. After collection, all data including laboratory results were checked for completeness by principal investigator each day and essential feedback were sent to data collectors. To ensure the accuracy of data, double data entry methods were used (49).

5.9.4 Data Analysais

Data were entered and analyzed using SPSS version 20. Descriptive statistics and logistic regression with bivariate and multivariate analysis were used to determine the association between dependant and independent variable with 95% confidence interval, P value <0.05 was considered as statistically significant to all test and results were presented by tables and figures.

5.10 Ethical Consideration

Ethical clearance was obtained from the Ethical Committee of School of Biomedical and Laboratory Sciences, College of Medicine and Health sciences, University of Gondar. Official permission and written informed consent was obtained from Borumeda Hospital, and study participants respectively. The consent of children was obtained from their family or guardian. All the information obtained from study participants were kept confidential. The laboratory result from the study participant was contacted to their doctors for appropriate treatment.

6. RESULTS

6.1 General characteristics of study participants

6.1.1 Socio-demographic characteristics

A total of 160 patients, majority were males 94 (58.8%), participated in this study. The mean (±SD) age of study participants was 55.11(± 17.85) years. In terms of educational status, most of the study participants were illiterate 132 (84.5%), majority 120 (75 %) were rural dwellers and their occupation was farmer 106 (66.3%) **(**Table1**)**.

6.1.2 Clinical characteristics

In this study, 69(43.1%) patients were suffering from conjunctivitis followed by blepharitis 47(29.4%). The dominant type of ocular infection among male patients (n=94) was conjunctivitis 41(43.6%) were as in female patients (n=66) a higher cases of dacryocystitis 7(10.6%) was observed (Figure 2).

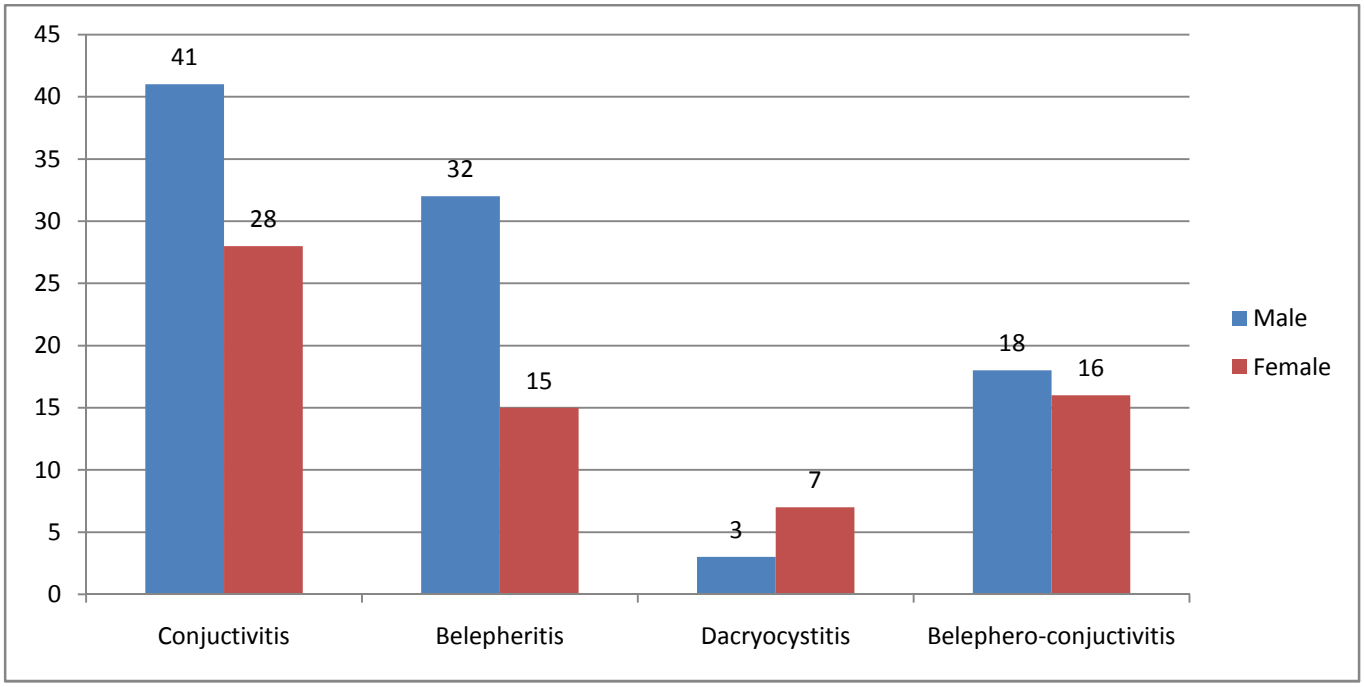

Figure 1-Clinical Features of External Ocular Infection in relation to Sex of Study Subjects at BH, Dessie, from February to May, 2014.

Table 1-Socio-demographic Characteristics of Patients with External Ocular Infections at BH from February to May, 2014.

Socio-demographic Characteristics	**(Frequency)**	**n (%)**
Age in years		
≤ 45	44	27.5
46-55	30	18.8
>56	86	53.8
Residence		
Rural	120	75.0
Urban	40	25.0
Sex		
Male	94	58.8
Female	66	41.3
Occupation		
Employed	14	8.8
Farmer	106	66.3
Merchant	11	6.9
Hose wife	14	8.8
Other*	15	9.4
Educational status		
Unable to write and read	132	82.5
Write and read only	26	16.3
primary school & above	2	1.3

Others *-student, daily laborer, unemployed, driver

6.2 Prevalence of the Bacterial Isolate

Among 160 ocular specimens subjected to cultures, 95(59.4%) were culture positive for different bacterial species. From these, 89(55.6%) had single species of bacteria and the remaining, 6 (4.4%) had two isolates. Majority (93.7%) of the isolates were Gram Positive bacteria; coagulase negative *Staphylococci* being the most frequent isolate 51(31.9%)

followed by *S. aureus* 21(13.1%) and *S.pneumoniae* 10(6.2%). *E. coli* 1(0.6%), *P. aeuroginosa* 1(0.6%), *K. pneumoniae* 1(0.6%), *P. mirabilis* 2(1.2%), *Salmonella* spp. 1(0.6%), and *Entrobacter* spp, 1(0.6%) comprised the negative isolates (Table 2).

Table 2-Bacterial Growth Pattern of Ocular Specimens from Study Participants at BH, Dessie from February to May, 2014.

Bacterial isolate	**Frequency**	**n (%)**
CoNS	51	31.9
S. areus	21	13.1
S. pneumonia	10	6.2
Viridian streptococci	3	1.9
S.pyogens	6	3.8
S. agalactiae	1	0.6
Enterococci spp	1	0.6
E.coli	1	0.6
p. aerogenosa	1	0.6
k. pneumonia	1	0.6
Proteus mirabilis.	2	1.2
Salmonella spp.	1	0.6
Enterobacter spp.	1	0.6
Total	**95**	**100**

6. 3 Association of Risk Factors and Bacterial Isolates

In this study, occupation, residence, education, frequency of face washing and the occurrence of systemic disease were used as possible risk and predisposing factors for ocular infection. The risk of acquiring in those patients with ≥ 56 years ages was 1.96 times more likely than those with ≤55 years age (OR=1.96, CI: 0.8-4.3). Patient that were less frequently washing their face for the bacterial ocular infection were 1.8 times more likely to be infected than the frequently washing (OR=: 1.8, 95% CI: 0.76-4.2). The risk of acquiring bacterial ocular infection in patient residence in rural was 1.4 times more likely than those in urban (OR=1.4, CI: 0.7-2.98).

Age, sex, residence, occupation, educational status, frequency of face wash, history of ocular trauma, past ocular surgery and systemic disease did not significantly ($P>0.05$) influence the occurrences of bacterial ocular infection in the current study through multivariate regression analysis. On the other hand, age and frequency of face washing were found to be significantly ($P<0.05$) associated with the occurrence of the bacterial ocular infection through bivariate logistic regression analysis (Table 3).

Table 3-Multivariate and bivariate logistic regression analysis of the identified predisposing risk factors and bacterial isolates from external ocular samples at BH, Dessie, Ethiopia from February to May, 2014.

Predisposing risk factors	**Bacterial isolate**		**COR*(95% CI)**	**P value**	**AOR*(95% CI)**
	Yes n(%)	**No n (%)**			
Age in years					
≤45	20(45.5)	24(54.5)	1		
46-55	17(56.7)	13(43.3)	1.57(0.62-3.996)	0.3	1.3(0.5-3.3)
≥ 56	58(67.4)	28(32.6)	2.49(1.2-5.2)	0.02	1.96(0.8-4.3)
Sex					
Male	57(60.6)	37(39.4)	1.14(.599-2.153)	0.7	-
Female	38(57.6)	28(42.4)	1		
Residence					
Urban	20(50)	20(50)	1		
Rural	45(37.5)	75(62.5)	1.7(.8-3.4)	0.2	1.4(0.7-2.98)
Occupation					
Employed	10(71.4)	4(28.6)	1		
Farmer	65(61.3)	41(38.7)	0.6(.9-2.2)	0.5	1.3(0.3-5.7)
House wife	5(35.7)	9(64.3)	0.2(.045-1.094)	0.06	0.5(0.2-1.5)
Other	15(57.7)	11(42.3)	.545(.135-2.2)	0.4	0.2(0.05-1.06)
Education					
Not write and read	80(60.6)	52(39.4)	1.5(0.09-25)	0.76	-
write and read only	14(53.8)	12(46.2)	1.2(0.07-21)	0.9	
Primary school & above	1(50)	1(50)	1		
Frequency of face washing					
More frequent	17(54.8)	14(45.2)	1		1
Frequent	16(41)	23(59)	.57(.22-1.5)	0.3	.7(.19-1.9)
Less frequent	62(68.9)	28(31.1)	1.82(.79-4.2)	0.2	1.8(.76-4.2)
History of ocular trauma					
Yes	9(64.3)	5(35.7)	1.26(.401-3.9)	0.7	-
No	86(58.9)	60(41.1)	1		
Past ocular surgery					
Yes	6(54.5)	5(45.5)	0.8(2.36-2.78)	0.7	-
No	89(59.9)	60(40.3)	1		
Systemic disease					
Yes	11(52.4)	10(47.6)	1.4(.55-3.5)	0.5	-
No	84(60.4)	55(39.6)	1		

*AOR=adjusted odds ratio, *COR=crude odds ratio

6.4 Antimicrobial Susceptibility Testing

In this study majority of Gram negative isolates were resistant to most of the antibiotics tested than the Gram positives. The rates of susceptibility of Gram positives range from 46.1% - 100 %. Among the Gram positives, 83(93.3 %) and 89(100 %) of the isolates were sensitive to cefoxitine and vancomycine respectively. However, more than half of Gram positives showed resistance against amoxicillin 48(53.9%) and 31(34.7%) were ampicyline resistant. Resistance to cefoxitine was observed among 4 isolates (7.8%) of the CoNS. *Viridian streptococci*, *S. agalactae* and *entrococci* isolates were 100% sensitive to all the drugs tested (Table 4).

Rates of susceptibility of Gram negatives range from 66.7 % - 83.3 %. The drug susceptibility patterns of the Gram negatives bacterial isolates showed that 5 out of 6 (83.3%) were sensitive to gentamycine. However, majority of Gram negative bacteria isolates 4 (66.7%) were resistance to tetracycline, norfloxacyline, ceftriaxone, and ciprofloxacin each. (Table 5).

Table 4-Antimicrobial Sensitivity Pattern of Gram Positive Bacterial Isolates from External Ocular samples at BH, Dessie, Ethiopia from February to May, 2014.

Organisms isolated(n=88)		**Antibiotics tested**								
		VAN	**PE**	**DO**	**AMP**	**AML**	**DA**	**FOX**	**CIP**	**CRO**
S.pneumoniae	S	10(100%)	6(60%)	8(80%)	7(70%)	4(40%)	9(90%)	9(90%)	8(80%)	10(100%)
	I	0	0	0	0	0	0	0	0	0
	R	0	4(40%)	2(20%)	3(30%)	6(60%)	1(10%)	1(10%)	2(20%)	0
CoNS	S	51(100%)	37(72.5%)	37(72.5%)	37(72.5%)	26(51%)	47(92.2%)	47(92.2%)	47(92.2%)	45(88.3%)
	I		0	1(2%)	0	5(9.8%)	0	0		
	R		14(27.5%)	13(25.5%)	14(27.5%)	20(39.2%)	4(7.8%)	4(7.8%)	4(7.8%)	6(11.8%)
S.aureus	S	21(100%)	14(66.7%)	12(57.1%)	11(52.4%)	7(33.3%)	17(81%)	20(95.2%)	19(90.5%)	20(95.2%)
	I	0	0	2(9.5%)	0	1(4.8%)	1(.6%)	0		
	R	0	7(33.3%)	7(33.3%)	10(47.6%)	13(61.9%)	3(14.3%)	1(4.8%)	2(9.5%)	1(4.8%)
S. pyogen	S	6(100%)	4(66.7%)	5(83.3%)	2(33.3%)	2(33.3%)	5(83.3%)	6(100%)	6(100%)	5(83.3%)
	I	0	0	0	0	0	1(16.7%)	0	0	0
	R	0	2(33.3%)	1(16.7%)	4(66.7%)	4(66.7%)	0	0	0	1(16.7%)
Enterococci spp.	S	1(100%)	1(100%)	1(100%)	1(100%)	1(100%)	1(100%)	1(100%)	1(100%)	1(100%)
	I	0	0	0	0	0	0	0	0	0
	R	0	0	0	0	0	0	0	0	0

CoNS*= Coagulase Negative Staphylococcus, S= sensitive, I= Intermediate, R= Resistance

Amp- Ampicyline, CRO- Ceftriaxone, FOX- cefoxitine , CIP- Ciprofloxacin, DA- Clindamycine, AMP - ampicyline, AML- amoxicillin ,PE- penicillin, VAN- Vancomycin

Table 5-Antimicrobial Sensitivity Pattern of Gram Negative Bacterial Isolates from External Ocular samples at BH, Dessie, Ethiopia from February to May, 2014.

Organism(n=6)	Sensitivity	Antibiotic tested				
		CRO	**NOR**	**TE**	**GS**	**CIP**
P. aerogenosa	S	1(100 %)	1(100%)	1(100%)	1(100%)	1(100%)
	I	0	0	0	0	0
	R	0	0	0	0	0
P. mirabilis	S	2(100%)	2(100%)	1(50%)	2(100%)	2(1000%)
	I	0	0	0	0	0
	R	0	0	1(50%)	0	0
E. coli	S	1(100%)	0	0	0	0
	I	0	0	0	0	0
	R	0	1(100%)	1(100%)	1(100%)	1(100%)
Salmonella spp.	S	1(100%)	1(100%)	1(100%)	1(100%)	1(100%)
	I	0	0	0	0	0
	R	0	0	0	0	0
Enterobacter spp.	S	0	0	1(100%)	1(100%)	0
	I	0	0	0	0	0
	R	1(100%)	1(100%)	0	0	1(100%)
k.pneumoniae	S	0	0	0	1(100%)	0
	I	0	0	0	0	0
	R	1(100%)	1(100%)	1(100%)	0	1(100%)

CIP- ciprofloxacyline , CRO- ceftriaxone, NOR-norfloxacyline , CIP- Ciprofloxacin, GS- Gentamycin, TE- Tetracyclin

S= sensitive, I= Intermediate, R= Resistance

6.4 Multi-drug Resistance Pattern of Bacterial Isolates from External Ocular Samples

Among the total bacterial isolates (n = 95) multi-drug resistance (MDR = resistance in ≥2 drugs) were recorded in 48 (50.5 %). In this study *S. aureus* accounts the highest drug resistance prevalence of 12(57%) then CoNS 28(54.9%).

Table 6-Multi-drug resistance pattern of isolates from external ocular spacemen's, at BH, from February to May, 2014.

Bacterial isolates	n	R1	R2	R3	R4	R5	Total
S. aureus	21	0	4(19.4%)	2(9.5%)	4(19.4%)	2(9.5%)	12(57%)
CoNS	51	5(9.8 %)	8(15.6%)	8(15.6%)	4(7.8%)	3(5.8%)	28(54.9%)
S. pneumoniae	10	0	2(20%)	1(10%)	2(20%)	1(10%)	6(60%)
S. pyogenes	6	0	1(16.6%)	2(33.3%)	1(16.6%)	0	4(66.6%)
E. coli	1	0	0	1(100%)	0	0	1(100%)
Enterobacter spp.	1	0	0	1(100%)	0	0	1(100%)
k. pneumoniae	1	0	0	1(100%)	0	0	1(100%)
Total		**5(5%)**	**15(15.8%)**	**16(16.8%)**	**11(11.6%)**	**6(6.3%)**	**53(58.3%)**

R1= resistant to one antimicrobial
R2= resistant to two antimicrobial
R3= resistant to three antimicrobial
R4= resistant to four antimicrobial
R≥5= resistant to greater or equals to five antimicrobial

7. DISCUSSIONS

The organisms that cause ocular infection are generally exogenous. However in certain circumstances they gain accesses to inter the eye and cause infection. In this study, prevalence of bacterial eye infection was 95(59.4%) of 160 ocular specimens for different bacterial species which are comparable with other studies done in Gondar 60.8% (51) and 54.2 % (40), Nigeria 50.3% (29) and in India 58.8% (17). However, the isolation rate was higher in Jimma 74.7 % (41), India 68.9% (52) and lower in Addis Ababa 47% (40). This may be due to difference in local climate, sample size, inclusion and exclusion criteria.

Gram positive bacteria were the dominant isolate accounting for 55.6% prevalence. This is also supported by other studies conducted in Ethiopia (42, 51), Nigeria (29), India (17) and other parts of the world had shows similar results inferring Gram positive cocci as a primary cause of bacterial external ocular infection.

Coagulase negative *Staphylococcus* was the most predominant pathogen with over all isolation rates of 51(31.9%). Comparable findings in India (5), Uganda (30) and Ethiopia (51) have been showed CoNS was the most predominant isolated pathogen. However, most of study shows that *S. aureus* was the predominant isolate (6, 29, 41, 42). The high pathogenicity of *S. aureus* is attributed to their being able to multiply and spread widely in tissues through their production of many extra cellular substances like coagulase which deposits fibrin on the surface of the microorganism altering their ingestion by phagocyte cells; alpha toxin (hemolysin) which lyse erythrocyte and damage platelets (49).

In Malaysia and Ethiopia *Streptococcus pneumoniae* was the commonest identified Gram positive organism in external ocular infection (32,34). We can understand from the above data at different settings that different types of Gram positive cocci bacteria could be particularly significant in causing ophthalmic infections. This variation may be due to differences in the environment, the standard of personal hygiene, age and site of infection.

The prevalence of Gram negative bacteria as causative agents of ophthalmic disease can be graded lower as only 6 (3.8%) were isolated. This was supported by study reports from Jimma (41), Southwestern parts of Ethiopia and in some African countries such as Nigeria

(6). The low prevalence of Gram negative enteric bacteria could be due to effective personal hygiene as the most important mode of transmission for enteric pathogens is fecal-oral contamination of the eye. During data collection, we noticed that health extension workers deliver health education about latrine usage and west disposal in the study area and the main cause for Gram negative ocular infection is contact lens wearer (28). No contact lens wearer was observed in the study area.

In the present study external ocular infections were predominantly seen in male sex due to their outdoor activities. This is similar to study in Gondar and Jimma (41, 51). The inter-age group variation in the three age groups was statistically not significant but, data showed that patients with ≥ 56 years of age has a higher frequency of 86(53.8%) of bacterial isolates. CoNS comprise higher prevalance 33(64.7%) than less age group. Similar study shows that *Staphylococcus epidermidis* and *Staphylococcus aureus* isolated greater percentage from the elders than children and adults (53). *Staphylococcus epidermidis* and *Staphylococcus aureus* is normal flora that parasitized conjunctival sac which is changing dynamically through our life time because of its long term exposure to the environment. Diseases will be caused when alteration of normal flora occurred. This alteration may be due to elders having a lower resistance from decreased immune functions, reduction of lacrimal secretions, reductions in some kinds of antibodies, complement proteins and enzymes in tears (54).

Study done by Long *et al* shows that relationship between positive culture of CoNS and different variables such as sex, age, composition of injury causing object and metallic injury ($p < 0.01$) were both significantly associated (56). Multivariate analysis of this study shows that bacterial isolate has no significant association with possible risk factor. The main reason may be since it is a hospital based study result may be accidental on the other hand sample size was small.

Resistance and sensitivity *in vitro* testing results clinician to provide information that allows making rationale based decision in choosing an initial regimen for treatment of ocular pathogens (13).

Based on results from susceptibility testing in this study, Gram positive cocci were 100% sensitive to vancomycine. However most of ampicyline and amoxicillin antibiotics were shows resistance. Similar findings have been reported in Gondar (51). This is because Vancomycin is basically a glycopeptides; which inhibits cell wall mucopeptide synthesis in early stages and it exhibited greatest potency against ocular Gram positive isolates (44). Reduced efficacy of ampicyline and amoxicillin could possibly due to the frequent usage of the drugs by the patients as these antibiotics are commonly used by many patients with and with no prescription. This is evidenced by the screeched establishment of drug venders which potentially sell antibiotics even with no direct order from the respective physician.

Most of Gram negative isolates were sensitive to gentamicin 5(83.3 %). However, majority of Gram negative bacteria isolates were resistance to tetracycline, norfloxacyline, ceftriaxon, ciprofloxacin 4(66.7 %). This is in agreement with similar study (55). *Pseudomonas* species tops the list of challenging organisms to treat because of high prevalence of resistant strains (45). In contrary in this study 100% sensitive to all antibiotics.

Resistance strain is increase in ocular bacterial isolate might be due to an irrational and unnecessary use of antimicrobial agents including the widespread use of systemic antibiotics which can result in the emergence of bacterial strains that show multidrug resistance. Other contributing factors may include improper dosage regimen during administration. This also includes because of difficulty of administration drops antibiotics in day time use in adult populations (16).

In Ethiopia, it is in common practice that antimicrobials can be purchased without prescription, which leads to misuse of antibiotics. This may contribute to the emergence and spread of antimicrobial resistance (40, 41, 42). Other factors may include availability of the suboptimal quality or substandard antimicrobial drugs, increased usage of a particular antimicrobial agent, poor sanitation, contaminated food and cross-contamination from humans or animals (6, 10).

8. LIMITATIONS OF THE STUDY

The aim of our study was on bacterial causative agents of external ocular infection, however, due to limitations anaerobic bacteria, *Chlamydia trachomatis* were not isolated. Some of the bacterial isolates were reported as CoNS which is not specific. Associated factor was not assessed for antimicrobial susceptibility test and sample size was too small.

9. CONCLUSIONS

This study shows that bacterial ocular infection is the major problem in Borumeda Hospital. The most commonly isolated bacteria were Gram positive organisms than Gram negatives. Coagulase negative *Staphylococci* were the predominant isolate followed by *S. aureus* and *S. pneumoniae*. Most of the Gram positive isolates were sensitive to vancomycin and cefoxitine, whereas Gram negative organisms were sensitive to gentamycine. However, a significant number of Gram negative bacteria isolates were resistance to tetracycline, norfloxacyline, ceftriaxone, ciprofloxacin and more than half of Gram positives showed resistance to amoxicillin.

10. RECOMMENDATIONS

Focusing attention on the present study results, the following recommendations are forwarded

- Science bacteria caused eye infection was the dominant problem observed in this study. Proper laboratory based diagnosis should be done to identify the etiology of the disease and antimicrobial susceptibility testing periodically to follow the change of pattern of the isolates and sensitivity over time.
- For Physicians
    - Decrease empirical therapy by, and advise isolation of causative bacterial agents, and subsequent susceptibility testing.
    - Most Sensitive drugs to bacterial isolate like vancomycin should be reserved for treating infections that are resistant to other anti-staphylococcal antibiotics or in cases of severe corneal infections.
- For individual
    - Avoid purchasing antimicrobials without prescription
    - Protect personal hygiene particularly face washing for which Strengthen the provision of contentious health education might be required from Borumeda Hospital.

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12. ANNEXES

Annex I Questionnaire (English Version)

Questionnaire for investigation of Bacterial Profile of external ocular Infections and their Antimicrobial Susceptibility Patterns in Patients Attending Borumeda Hospital: Northeast Ethiopia.

Questionnaire code number	
Card number	
Date of data collection	
Diagnosis	

Socio-demographic information

Instruction: Read the questions audible and circle the appropriate response; for question 1, enter age in years.

SN	QUESTIONS	CODING CATEGORIES
1	**Age(in years)**	
2	Sex	1.Male 2.Female
3	Education status	1. Civil servant 2. Farmer 3.Merchant 4.House wife 5. Daily laborer 6. Other (specify)_____
4	Residence?	1. Urban 2. Rural
5	Occupation?	1. Civil servant 2. Farmer 3.Merchant 4.House wife 5. Daily laborer

		6. Other (specify)_____
6.	Type of ocular infection?	1. Blepharitis 2. Conjunctivitis 3. Belpharo- Conjunctivitis 4. Hordeolum 5. Dacryocystitis 6. Other infection, specify
7	Was antibiotic prophylaxis given?	1.Yes 2.No
8	Have you get eye trauma?	1.Yes 2.No
9	Do you wear contact leans?	1.Yes 2.No
10	Do you perform eye surgery?	1.Yes 2.No
11	Availability of chlorinated water source?	1.YES 2.NO
12	Frequency of face wash in one day	1. more frequent 2. frequent 3. less frequent
13	Have you ever had a blood test for diabetic mellitus?	1.Yes 2. No
14	If yes, what was the result?	1. I am known diabetic patient 2. I am normal
15	Do you have Systemic infection?	1. Yes 2. No

THANK YOU!!

Annex -II Laboratory Data Collection Form

1. Code no_____

2. Age _____Sex _____ Date_____

3. Types of specimen swab of ulcer, purulent specimen, vitreous aspiration & corneal scrapings

4. Media used_____

5. Organism isolated

_____

_____

_____

Culture and biochemical tests identification

_____

_____

_____

_____

7. Gram stain from specimen_____

8. Result of Gram stain from culture_____

9. Antimicrobial test

Sensitive to _____

Intermediate to_____

Resistance to_____

Comments

Name of principal investigator _____

Signature _____ Date_____

Annex- III Procedure External ocular infections

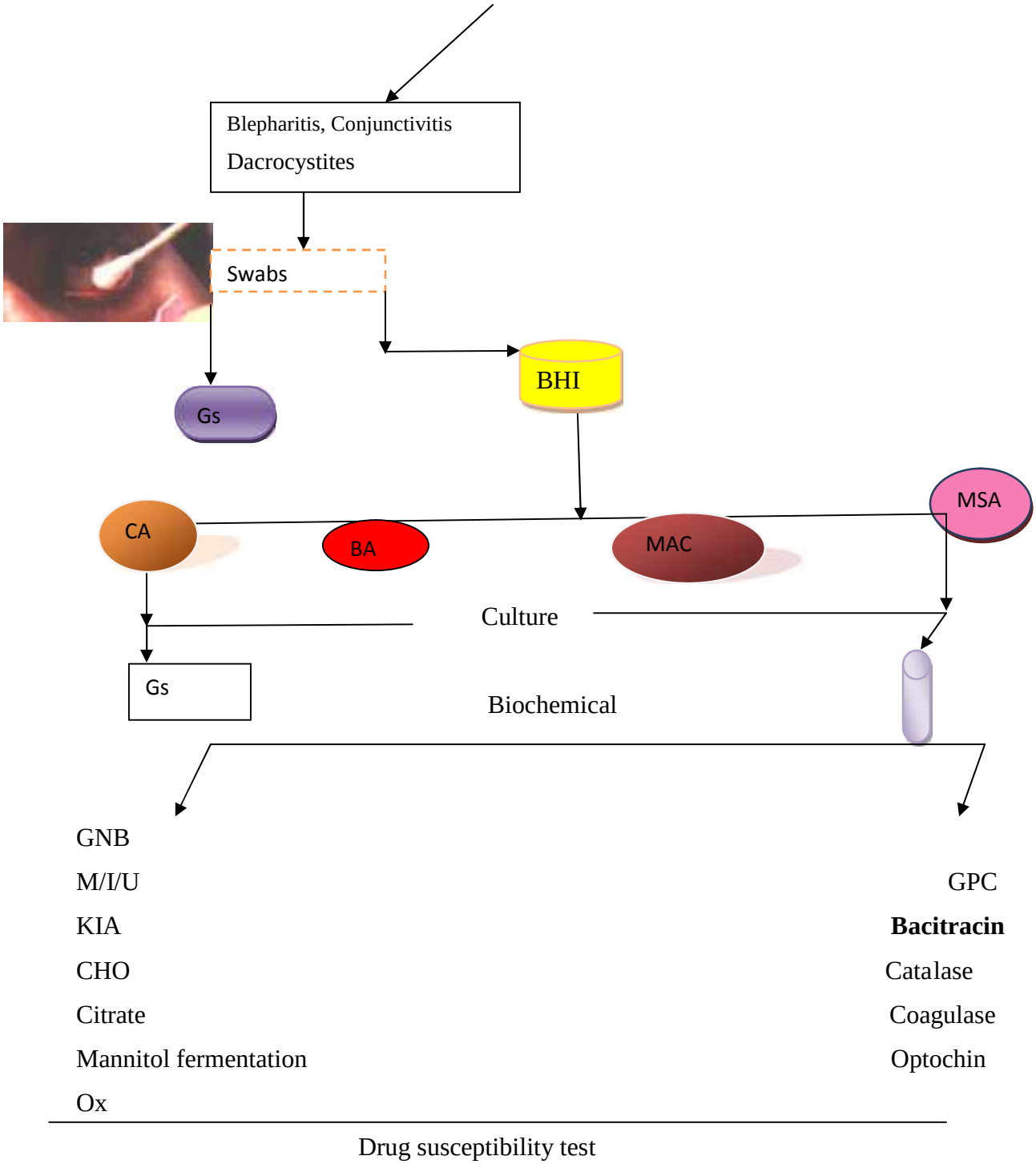

Figure 2 Flow Chart for Laboratory Procedur**Terms:** GPB=Gram positive bacilli, GNB= Gram negative bacilli, M/I/U= Motility/Indole/Urea, Ox= Oxidase test, CHO= Carbohydrate Utilization tests, BA= Blood agar, CA= Chocolate agar, MSA= Manitol salt agar, MAC= MacConkey agar, BHI=Brain Heart Infusion transport mediu

A. To prepare the culture media

1. Read the label on a bottle of dehydrated agar media. It specifies the amount of dehydrated powder required to make 1 liter (1,000 ml) of medium. Calculate the amount needed for 1/2 liter and weigh out this quantity.
2. Place 500 ml of distilled water in an Erlenmeyer flask. Add the weighed, dehydrated agar while stirring with a glass rod to prevent lumping.
3. Set the flask on a tripod over an asbestos mat.
4. When the agar mixture was completely dissolved, remove the flask from the flame or hot plate, close it with the cotton plug or cap, and it has to be sterilized in the autoclave.
5. When the flask of sterilized agar was returned to you, allow it to cool to about 50°C (the agar should be warm and melted, but not too hot to handle in its flask). Remove the plug or cap with the little finger of your right hand and continue to hold it until you are sure it won't have to be returned to the flask. Quickly pour the melted, sterile agar into a series of petri dishes. The petri dish tops are lifted with the left hand and the bottoms are filled to about one-third capacity with melted agar.
6. Replace each petri dish top as the plate was poured. When the plates are cool (agar solidified), invert them to prevent condensing moisture from accumulating on the agar surfaces.
7. Place inverted agar plates in the 35°C incubator. They should be incubated for at least 24 hours to ensure they are sterile (free of contaminating bacteria) before you use.

B. Collection and processing of specimen from ocular infection

I. The specimens were collected by an experienced optometrist. Special care was taken to avoid contaminating the specimen with commensal organisms from the skin by swiping normal saline moistened cotton.

II. With sterile cotton tipped applicator stick moistened with normal saline collect sample from the infected site by rubbed over the lower conjunctival sac from medial to lateral side and back again, swabbing the lid margins in cases of blepharitis. Purulent material in dacryocystitis was collected by everte dpuncta then applying pressure over the lacrimal sac area.

III. Label the sample with the patient code number

IV. Inoculate in to checolate, MacConkey and blood agar manitol salt agar aseptically

V. Incubate the plate aerobically at 35-37 ℃for 18-24 hours.

VI. Examine and report the culture; look for colony characteristics and perform biochemical test.

VII. Determine drug susceptibility pattern of the isolated organism

C. Gram stain procedures

1. Prepare a thin smear of the culture or specimen will be observed.
2. Allow to air-dry and fix the smear.
3. Cover the fixed smear with crystal violet for 1 min.
4. Rinse with clean water and tip off all the water.
5. Cover the smear with Lugol's iodine for 1 min.
6. Wash off the iodine with clean water.
7. Add acetone-alcohol for 30 sec.
8. Wash the smear immediately with clean water.
9. Cover the smear with saffranin for 1-2 minutes.
10. Rinse with clean water.
11. Wipe the back of the slide and place in a draining rack for the smear to air-dry.
12. Examine microscopically, first with the 40x objective and then with the oil immersion objective for white cells, bacteria and other structures.

- Gram- positive bacteria ----------Dark purple
- Gram- negative bacteria ----------Pale to dark red.

D. Biochemical testing procedures

Identification of Gram positive bacteria: Gram positive cocci were identified based on their

Gram reaction, catalase and coagulase test results.

Catalase test: This test was used to differentiate *staphylococci* (positive) from *streptococci (*negative)

Procedure

A. Pour 2-3 ml of 3% hydrogen peroxide to a test tube
B. Using a sterile wooden stick take the test organism and immerse into the hydrogen peroxide solution
C. Look for immediate bubbling

Interpretation:

- Active bubbling…….. positive test
- No release of bubbles……negative test

Coagulase test: This test was used to differentiate *Staphylococcus aureus* from other *Staphylococcus* species.

Procedure

I. Place a drop of physiological saline on two separate slides
II. Emulsify the test organism in each of the drop to make thick suspension
III. Add one drop of plasma to one of the suspensions and mix gently. Look for clumping of the organism within 10 seconds

Interpretation:

- Clumping within 10 seconds ----------*S.aureus*
- No clumping within 10 seconds ----------other staphylococcus species

Identification of Gram negative bacteria: was based on their test result with a series of biochemical tests.

Procedure

1. Prepare a suspension of the test organism with nutrient broth 3-4 colony of test organism in 5 ml nutrient broth.
2. A loop full of the bacterial suspension was inoculated in to indole, citrate agar, triple sugar iron agar, lysine decarboxylase agar, manitol, urea agar and motility medium.
3. Incubate at 35-37 ^{O}c for 18-24 hours.
4. Look for color change (turbidity for motility) of the medium
5. Identify the test organism by considering the result of the six biochemical tests

E. Antimicrobial susceptibility testing

Procedure

1. Prepare a suspension of the test organism by emulsifying several colony of the organism in a small volume of nutrient broth
2. Much the turbidity of suspension with turbidity standard
3. With a sterile swab take sample from the suspension (squeeze the swab against the side of the test tube to remove the excess fluid).
4. Spread the inoculums evenly over the Muller-Hinton agar plate with the swab.

5. Using a sterile forceps or needle, place the antimicrobial disc on the inoculated plate
6. Incubate the plate aerobically at 35-37^{o}C for 18-24 hours
7. Read the test after checking that the bacterial growth is neither heavy nor light. Measure the radius of the inhibition zone.
8. Interpret the reaction of the test organism to each antibiotics used as sensitive, intermediate or resistance, using the standard.

- Sensitive – zone of radius is wider or equal to the control.
- Intermediate –zone of radius is more than three mm smaller than the control
- Resistance – no zone of inhibition.

Annex-V Information Sheet and Consent Form

Title: Bacterial profile of external ocular infections and their antimicrobial susceptibility pattern in patients attending Borumeda Hospital North East Ethiopia, 2014.

Name of Investigator: Birtukan Shiferaw

Name of the Organization: University of Gondar Teaching Hospital

Introduction: You are invited to participate as a study participant in a research conducted. Your participation is voluntarily. The research team includes principal investigator, data collector and supervisor.

Purpose: -The main objective of the study is to collect information on external ocular infection and susceptibility pattern for antimicrobial agents in and out patients.

Procedure to be carried out: In order to perform the indicated study at the Dessie town you are invited to take part in this project. If you are willing to participate, you need to understand the purpose of the study and your consent. The required clinical sample will be collected by ophthalmic nurses and ocular surgeons who are currently working in the eye unit. Then, you are requested to give your consent to the sample collector.

Risk associated with the study - You will not be at any physical or psychological risk and should experience no discomfort resulting from the research procedures but during collection of the pus you may feel some discomfort, this does not produce serious pain.

Benefits of the study - Based on the diagnosis result you will be treated accordingly. Moreover, this study will have a great value on preventive measures in hospitals and in the community. The results of this study have importance to treat the patients and to use as a baseline for effective treatment in the absences of laboratory investigation.

Compensation for participation: You will not receive any payment for your participation in this research study.

Confidentiality of your information- All information gathered from the study participant will remain confidential. Your participation in this study is strictly anonymous. Personal information will be treated confidentially and under no circumstances will it be transmitted to any person or organization. The results of this study will be evaluated and summarized, and a feedback of the results to the study participants will be given by principal investigator.

Voluntary participation - Your participation in the study is absolutely voluntary; no one is obliged to take part. Refusal to participate will involve no penalty. Each study participant is free to withdraw consent and discontinue participation in this study at any time.

Alternative option: - If you are not interested in this study you can leave it and you will do in the main laboratory.

Person to contact: If you have any question you can contact the principal investigator and you may ask at any time you want.

Principal investigator: **Birtukan Shiferaw** Cell phone +251913120877

E-mail: birtukan_sheferaw@yahoo.com

Consent form

Serial no---------- Name of health institution- Borumeda Hospital

Card no ---------- Date----------

I the undersigned study participant with ocular infection have been well informed about the objective of the study entitled “Bacterial isolates and Antimicrobial susceptibility patterns of external ocular infection in Borumeda Hospital in Dessie Town Northeast Ethiopia, 2014".

I am also told that all the information obtained at any course of the study is to be kept confidential. Moreover I have also been well informed of my right to keep hold of, decline to cooperate and drop out of the study if I want and none of my actions will have any bearing at hospital access. I agreed voluntarily to provide the requested samples from me as well as my child.

Name and signature of study participant_____ Date_____

Name and signature of investigator _____Date_____

Annex –VI Questionnaire (Amharic Version)

በበሽታ በተጠቃ ዐይን ዉስጥ የሚገኙ ባክቴሪያዎችን መለየትና የጸረባክቴሪያ መድሀኒት የመቋቋም ባህሪያቸዉን ለማጥናት የተዘጋጀ መጠይቅ

የኮድቁጥር	
የመታከሚያካርድቁጥር	
ናሙናዉየተወሰደበተቀን	
ምርመራ	

ሀ. ማህበራዊና ኢኮኖማዊ መረጃ

መመሪያ፡- አያንዳንዱን ጥያቄዎቹን ከነበባችሁ በሀላ ከአማራጮቹ ውስጥ የተሰጠውን መልስ በማክበብ ለጥያቄ ቁጥር አንድ መልሱን በቁጥር ጻፉ::

ተራቁጥር	ጥያቄ	ኮድ
1	እድሜ ?	
2	ጾታ	1.ወ 2.ሴ
3	የትምህርት ደረጃ	1.ያልተማረ 2.የተማረ
4	መኖሪያ ቦታ	1. ገጠር 2. ከተማ
5	ስራ	1.የመንግስት ሰራተኛ 2.ገበሬ 3.ነጋዴ 4.የቤት እመቤት 5.የቀን ስራ 6.ሌላ ካሉ ይግለፁ
የአይን ሁኔታ		

6	በየትኛዉ አይነት የአይን በሽታነዉ የተጠቃው ?	1.Blepharitis 2.Conjunctivitis 3.Keratitis 4.Hordeolum 5.Dacryocystit 6.Other infection, specify
የታካሚዉ ሁኔታ		
7.	የአይን በሽታ መከላከያ መድሃኒት ተሰጥቶሀል/ሻል;	1. አዎ 2.አልተሰጠኝም
8.	ፊትሽ/ህን በቀን ምን ያህል ጊዜ ትታጠቢያለሽ/ባለህ ?	1.በተደጋጋሚ 2. አልፎ አልፎ 3. በጣም በተደጋጋሚ
9.	ንፁህ የመጠጥ ውሀ በአቅራቢያዎ አለ?	1.አዎ 2.የለም
10.	የደም ዉስጥ የስኳር መጠን ተመርምረዉ ያዉቃሉ?	1. አዎ 2. አልተመረመርኩም
11.	አዎ ካሉ	1.የስኳር በሽተኛ ነኝ 2. ጤነኛ ነኝ
12.	ሌላ ተጨማሪ በሽታ አለብዎት?	1.አዎ 2.የለም
13.	አዎ ካሉ ምን ነበር?	
14.	አይን ላይ አደጋ ደርሶቦወት ያውቃል ?	አዎ አያውቅም
15.	የአይን ቀዶ ጥገና ተሰርቶልሽ/ህያውቃል ?	አዎ አያውቅም
16.	የአይን (contact lens) አድርገሽ /ህታውቂያለሽ/ህ?	አዎ አላውቅም

ለትብብርዎ እጅግ አድርገን እናመሰግናለን!!!

Annex VII የጥናቱ ማብራሪያ /Information Sheet/

የጥናቱ ርእስ፡

በበሽታ በተጠቃ ዐይን ዉስጥ የሚገኙ ባክቴሪያዎችን መለየትና የጸረባክቴሪያ መድሀኒት የመቋቋም ባህሪያቸዉን በደሴ ከተማ በሚገኘው ቦሩሜዳ ሆስፒታል ለማዎቅ ነዉ፡፡

ጥናቱን የሚያካሂደዉ ሰዉ - ብርቱካን ሽፈራው

ለጥናቱ ተሳታፊዎች /ወላጆች/አሳዳጊዎች የሚሰጥ ማብራሪያ"

መግቢያ:- በመጀመሪያ እርስዎ በዚህ ጥናት ዉስጥ ተሳታፊ እንድሆኑ ሲጠየቁ ተሳታፊ የሚሆኑት ፈቃደኛ ከሆኑ ብቻ ነዉ፡፡ ይህንን ጥናት የሚያካሂዱት ሰዎች የተዋቀሩት በዋና ተመራማሪ፣ በጥናቱ ተቆጣጣሪዎች እና ናሙና ሰብሳቢዎች ነዉ፡፡

ዓላማ፡- የዚህ ጥናት ዋና አላማ በበሽታ በተጠቃ ዐይን ዉስጥ የሚገኙ ባክቴሪያዎችን መለየትና የጸረባክቴሪያ መድሀኒት የመቋቋም ባህሪያቸዉን ለማወቅ ነዉ፡፡

ለጥናቱ የሚያስፈልግ ናሙና አወሳሰድ፡- ይህን ጥናት በደሴ ከተማ በሚገኘው ቦሩሜዳ ሆስፒታል ለማካሄድ የእርስዎን ግለ-ታሪክ መጠየቅና ፈቃደኛነትዎን ማግኘት አስፈላጊ ነዉ፡፡የጥናቱን አላማና ጥቅም በሚገባ ተረድተዉ ለመሳተፍ ፍላጎት ካለዎት፣

ሀ. ናሙና ለሚዎስደዉ ሰዉ ወይም ዋና ተመራማሪ ለሚጠይቀዎት ጥያቄ ተገቢዉን መልስ ይሰጣሉ፡፡

ለ. ለምረመራ የሚያስፈልገዉን ናሙና ይሰጣሉ፡፡

ከጥናቱ ጋር ተያይዞ የሚመጣ ጉዳት - በዚህ ጥናት ዝርዝር አሰራር ሂደት ውስጥ አካላዊ ወይም አእምራዊ ጉዳት አይኖርም፡፡ ነገርግን ናሙናዉ በሚወሰድበት ጊዜ መጠነኛ የሆነ የህመም ስሜት ሊሰማወት ይችላል፡፡ይህ የህመም ስሜት ምንም አይነት ችግር አያመጣብዎትም፡፡

ለተሳትፎ የሚሰጥ ማካካሻ ፡ምንም አይነት የካሳ ክፍያ የለዉም፡፡

የጥናቱ ጥቅም፡ የምርምሩ ዉጤት በዐይን ዉስጥ የሚገኙ አደገኛ የሆኑ ባክቴሪያዎችን በማጥናት የመፈወስ አቅምያላቸዉን መድሀኒቶችን ለማወቅ ያስችላል፡፡ እንደዚሁም በእርስዎ ዐይን ላይ እነዚህ ባክቴሪያዎች ከተገኙ አስፈላጊዉ መድሀኒት እንዲያገኙ ለሃኪሙ ይነገርሎታል፡፡በተጨማሪም ይህ ጥናት የመከላለል ስራ በሆስፒታል እና በህብረተሰቡ ዘንድ እንዲኖር ያግዛል፡፡እንደዚሁም ደግሞ የላቦራቶሪ አገልግሎት በሌለበት ጤናተቋም ትክክለኛ መድሃኒት ለመስጠት እንደአመላካች ሆኖ ያገለግላል፡፡

የመረጃሚ ስጢራዊነት -ሁሉም ከተሳታፊዎች የሚሰበሰቡ መረጃዎች በሚስጢር የሚያዙ እና የሚጠበቁ ይሆናሉ፡፡በማንኛውም ምክኒያት ተሳታፊዎች እነማን መሆናቸውን የሚያሳይ በመጠይቁ ይሁን በሌላነገር አይኖርም፡፡የተሰበሰቡ መረጃዎች ለሶስተኛ ወገን ተላልፎ አይሰጥም፡፡በተጨማሪም ውጤቱ የሚለካዉ ይሁን ተሰብስቦ የሚያዘው በዋና አጥኚ ነው፡፡

በፍቃደኝነት ላይ የተመሰር ተተሳትፎ - በጥናቱ ለመሳተፍ ሙሉ በሙሉ በተሳታፊዎች ፍቃደኝነት የተመሰረት ነው፡፡ከዚህ በተጨማሪ የተሳታፊዎች መሳተፍ እና አለመሳተፍ በራሳቸዉ ብቻ የሚወሰን እንጂ የማንም ጣልቃ ገብነት አይኖረውም፡፡እንደዚሁም ደግሞ ተሳታፊዎች በማንኛውም ግዜያ ለምንም ቅጣት ጥናቱን ማቋረጥ ይችላሉ፡፡

አማራጭ -ማንኛዉም በጥናቱ መሣተፍ ፍላጎት የሌለዉ ከዋናዉ በዞኑ በሚገኘው ላቦራቶሪ ሄዶ ማሰራት ይችላል፡፡

ተጠሪ - በማንኛዉም ጊዜ መጠቅ የሚፈልጉት ጥያቄ ካለ መጠየቅ ይችላሉ፡፡

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የስምምነት ዉል

ቀን----------

ተራቁጥር----------የሚታከምበት ክፍል---------- የካርድ ቁጥር----------

እኔ ከዚህ በታች ስሜ የተጠቀሰዉና የፈረምኩት የጥናቱ ተሳታፊ በዐይን ዉስጥ የሚገኙ ባክቴሪያዎችን መለየትና የጸረባክቴሪያ መድሀኒት የመቋቋም ባህሪያቸዉን በደሴ ከተማ በሚገኘው ቦሩሜዳ ሆስፒታል ለማዎቅ የሚደረገዉን ጥናት አላማና ጥቅም በሚባ ተረድቻለሁ፡፡ ጥናቱ ላይ መሳተፍም ሆነ አለመሳተፍ በራሴ ፍቃድ የሚወሰን መሆኑም ተገልጾልኛል፡፡ በተጨማሪም ከጥናቱ ባልሳተፍም ሆነአቋርጬ ብወጣ ጤናተቋሙ በማገኘዉ የህክምና አገልግሎት ምንም አይነት ችግር እንደማይደርስብኝ ተነግሮኛል፡፡

በመሆኑም ከእኔም ሆነ ከልጄ አይን ናሙና ተጠሪነ መዉሰድ አስፈላጊ መሆኑን ስለተስማማሁበት ለመስጠት ሙሉ ፈቃደኛ መሆኔን በፊርማዬ እገልጻለሁ፡፡

የተሳታፊ ስምና ፊርማ _____ቀን _____

የተመራማሪ ስምና ፈርማ _____ ቀን _____